

Homology Arms size (L/R)	ssDNA Oligo Sequence	% Sequence Integration (Pos/Total)
20/18	ACCTCCTCTGGAACCTGGAC GATATCC CACGGGCATCTGTGACTG	57 (42/74)
55/18	CAGCAATTTCTACTACGGTTGT CAGTACGCAACGCACGTCGTC AGGAACCTGGAC GATATCC CACGGGCATCTGTGACTG	5 (2/41)
23/48	CGGACCTCCTCTGGAACCTGGAC GATATCC CACGGGCA ACTCTGACAGTTCT TCGGATATGAGCACTTGTAAGTCACC	0 (0/20)
48/45	TTCTACTACGGTTGTGAGCACCCAGCGGACCTCCTCTGGAACCTGGAC GATATCC CACGGGCATCTGTGACTGCTGTTCTGATATGAGCACTTGTAAGTC	0 (0/155)
20/18	ACCTCCTCTGGAACCTGGAC ATAACTTCGTATAGCATACATTATAGCAATTTAT CACGGGCATCTGTGACTG	55 (89/162)
55/18	CAGCAATTTCTACTACGGTTGT CAGTACGCAACGCACGTCGTC AGGAACCTGGAC ATAACTTCGTATAGCATACATTATAGCAATTTAT CACGGGCATCTGTGACTG	35 (17/48)
23/48	CGGACCTCCTCTGGAACCTGGAC ATAACTTCGTATAGCATACATTATAGCAATTTAT CACGGGCA ACTCTGACAGTTCT TCGGATATGAGCACTTGTAAGTCACC	8 (4/48)

EcoRV: GATATCC

mLoxP: ATAACTTCGTATAGCATACATTATAGCAATTTAT

Bold: Sequence mutation to prevent TALEN binding

Supplementary Table 1: ssDNA oligos designed to test for homology directed repair (HDR). Different length of homology arms to the *ponzr1* locus were tested. The first four sequences incorporated EcoRV into the genome (red). The EcoRV integration was tested using a semi-quantitative PCR plus restriction enzyme digest method. The last three conditions detect the engineered mloxP using a standard PCR method with the reverse primer using the mLoxP sequence. The homology arms of the first (20/18) go halfway through the TALEN binding site and showed significant HDR using both EcoRV (red) and mLoxP (blue) oligos. When the homology arms were lengthened to include the entire TALEN binding site (48/45), no HDR was seen and a decrease in TALEN cutting was noted (data not shown). To prevent potential binding of the TALENs to the ssDNA oligo, mutations were introduced to one side of the longer homology arms (bold) and the other side was truncated halfway through the binding site. The long homology arms with mutations were made for either the 5' (55/18) or 3' (23/48) end. However, both these oligos demonstrated significantly less sequence incorporation when compared to the 20/18 sequence (top). The final column shows percent of somatic sequence integration with the number of positive embryos versus total embryos tested in parenthesis.